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Synergistic treatment of cancer stem cells by combinations of antioncogenes and doxorubicin

Jie Chen [a, 1], Lin Lin [a, 1], Zhaopei Guo [a], Caina Xu [a], Huayu Tian [a, *], Kinam Park [b], Xuesi Chen [a]

[a] Key Laboratory of Polymer Ecomaterials, Changchun Institute of Applied Chemistry, Chinese Academy of Sciences, Changchun 130022, China
[b] Departments of Biomedical Engineering and Pharmaceutics, Purdue University, West Lafayette, IN 47907, USA

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ABSTRACT

The combined treatment of two or more therapeutic approaches has been demonstrated to be an efficient strategy for cancer stem cells (CSCs) therapy. A new strategy for treatment of CSCs, which have a high self-renewal capacity, is introduced by combination of antioncogenes and doxorubicin (DOX). Cancer cell-associated genes (MDR1 and Sox2) are proved to be up-regulated in CSCs, resulting in increased drug resistant compared with normal cancer cells. In this work, the multidrug resistance of CSCs is reversed by down regulation of the over-expressed cancer cell-associated MDR1 and Sox2 gene, and the enhanced DOX endocytosis is achieved. In addition, the combined therapeutic approach resulted in improved therapeutic effect of CSCs. The combination therapy of CSCs with the down regulation of multidrug resistance and a therapeutic antitumor drug can be a useful strategy in cancer therapy.

1. Introduction

Cancer is one of the most lethal diseases affecting the human life worldwide [1]. Currently, many strategies have been successfully used in the antitumor treatment [2–7]. The conventional cancer therapies include surgery, radiation, hormonal treatment and chemotherapy [8,9]. Among them, chemotherapy is highly effective for treatment of cancers. Chemotherapy, however, is effective only during the early-stage treatment. Eventually, drug resistance and recurrences with more distant metastases occur leading to chemotherapy failure [10]. Usually, such recurrences are resistant to conventional anticancer therapies, which is mainly due to the presence of cancer stem cells (CSCs) [11]. CSCs comprise a rare subpopulations (usually<~1%) in tumors, while exhibit stem cell-like properties allowing them to initiate the tumor growth and metastasis [12]. They are highly drug resistant and able to differentiate into multi-lineages of resident cells in tumors to re-initiate the growth of tumors. This may be a reason for tumor recurrence post conventional therapies [13]. CSCs were first isolated from acute myeloid leukemia by Bonnet and Dick in 1997 [14]. Since then CSCs have been identified in many types of human common tumors including brain [15], breast [16], colon [17], hepatoma [18], and pancreatic carcinomas [19]. There are many biomarkers especially expressing in CSCs, such as homeobox transcription factor Nanog (Nanog), transcription factor SOX2 (Sox2), myc proto-onco-gene protein (C-myc), octamer binding protein 4 (Oct4), CD44, CD133 and so on [20,21]. CD44 is a hyaluronan-binding glycoprotein, and most commonly used for the isolation of CSCs by combined with other markers in many kinds of tumors [22,23]. The biomarkers can be used to analyze CSC populations by immunohistochemistry and reverse-transcriptase polymerase chain reaction (RT-PCR) [22].

The key factor of eradicating tumor cells is the effective killing of all CSCs, and the innate drug resistance feature of these cells is a main obstacle in cancer treatment [16]. In recent years, several promising strategies have been developed for treatment of CSCs [24]. Targeted therapy of CSCs plays an important role in cancer therapy [25]. Thioridazine (THZ) was reported to be selectively targeted cancer stem cells, but no effect on normal cells, which was mainly due to significantly higher expression of THZ antagonized dopamine receptors in CSCs [11]. A combination therapy was contributed to the therapy of pancreatic CSCs by blockading of mTOR signaling and sonic hedgehog together with rapamycin, cyclopamine and gemcitabine [26]. Reversing the drug resistance of

* Corresponding author.
E-mail address: thy@ciac.ac.cn (H. Tian).
[1] These authors contributed equally to this work.

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CSCs is another important strategy to enhance the efficacy of chemotherapy. It is well known that several genes are up-regulated within CSCs, which constitute unique properties and maintain the activity of CSCs [27]. The sensitivity of the conventional drugs is expected to be improved by reducing over-expressed genes, such as multidrug resistance gene (MDR1), transcription factor SOX2 (Sox2), myc proto-oncogene protein (C-myc) [22]. Thus, RNA interference (RNAi) gene silencing technology would play an important role in tumor therapy by knocking down the expression of specific genes in CSCs [20]. Because the CSCs populations are highly complex and heterogeneous, combination therapeutic approaches may be necessary for effective cancer treatment.

Over-expression of Sox2 is acritical factor in maintaining the pluripotent property and drug resistance of CSCs. In this study, a new strategy for treatment of CSCs was developed by combined delivery of a therapeutic plasmid that expresses interfering RNA targeting Sox2 (shSox2) and doxorubicin (DOX), one of the most effective anticancer drugs. The approach taken in this study is co-delivery of carrier/shSox2 complexes for down-regulation of the Sox2 gene expression and DOX for killing tumor cells. It is proposed that down-regulation of multidrug resistance of CSCs and cell apoptosis induced by DOX results in better results in clinical anti-tumor therapy (Scheme 1).

2. Materials and method

2.1. Materials

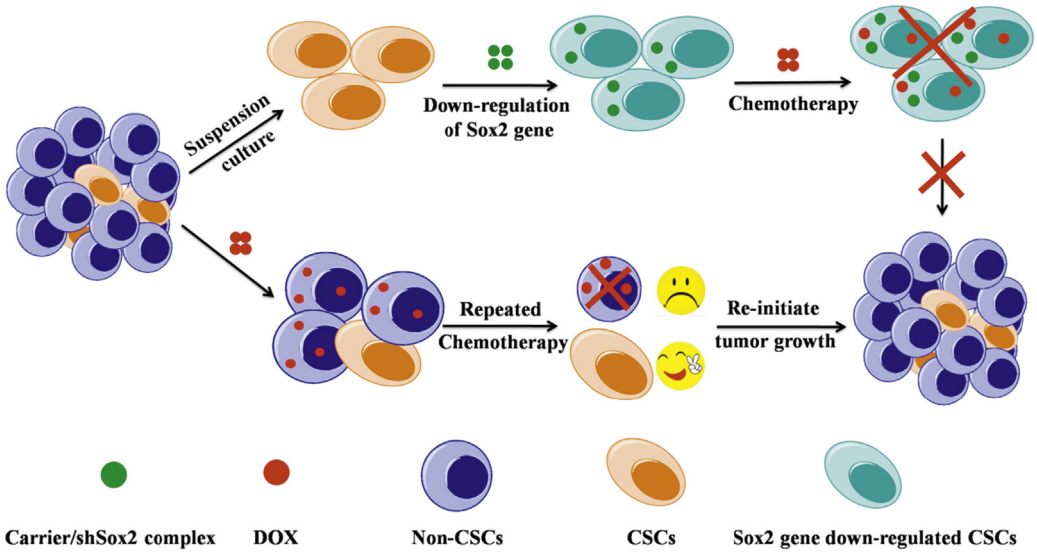
Branched polyethylenimine with average molecular weight of 25 kDa (PEI-25k) was purchased from Aldrich. GoldenTranfer reagent was obtained from Changchun GoldenTransfer Science and Technology Co. Ltd. (Changchun, China) Dulbecco's Modified Eagle Media: Nutrient Mixture F-12 (DMEM-F12) was purchased from Gibco (Grand Island). B27 was purchased from Invitrogen (Carlsbad, USA). Human Epidermal Growth Factor (hEGF) was purchased from BD Biosciences (New Jersey, USA). Low-endotoxin bovine serum albumin (BSA) was purchased from Sangon Biotech (Shanghai, China). Insulin was purchased from Sigma–Aldrich Chemical Co. (St. Louis, MO). 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) was purchased from Amresco (Solon, Ohio, USA). A plasmid that expressed interfering RNA targeting Sox2 and siRNA duplexes were purchased from GenePharma Co. Ltd. (Shanghai, China) and the target sequences were designed as follows: shSox2: CGCTCATGAAGAAGGATAAGT, and the control sequence (GTT CTC CGA ACG TGT CAC GT) does not match any human genome sequence. MDR1: sense: 5′-GGAAAA-GAAACCAACUGUCdTdT-3′, antisense: 5′-GAC AGU UGG UUU CUU UUC CdTdT-3'.

2.2. Mammosphere culture

Human breast adenocarcinoma cells (MCF-7 cells) were cultured in suspension in serum-free DMEM-F12 at 1.5×10^3 cells/mL in an ultra-low attachment culture flask, and the medium was supplemented with B27 (1:50, v/v), 20 ng/mL hEGF, 0.4% low-endotoxin BSA, and 5 mg/mL insulin. During the process of forming mammospheres, the cell culture flask should be shaken gently everyday to prevent the adherence of the cells. After incubation for 14 days, the mature mammospheres were harvested by gentle centrifugation, dissociated into single cells as previously described. To prepare mammospheres, the single cells were cultured to proliferate to the next generation of mammospheres.

2.3. Drug resistant activity in CSCs

To evaluate the drug tolerance of MCF-7 mammosphere cells, various concentration of DOX were assessed by the MTT viability assay, MCF-7 adherent cells were used as the control. Briefly, MCF-7 mammosphere single cells were seeded in ultra-low attachment 96-well plates at a density of 1.0×10^4 cells per well in 100 μL of serum-free DMEM-F12 at 37 °C in 5% CO_2 for 24 h prior to the treatment of DOX with the concentration range from 0.16 μg mL^{-1} to 10 μg mL^{-1}. At 48 h post-treatment, 20 μL of MTT stock solution (5 mg mL^{-1} in PBS) was added to each well. After incubation for an additional 4 h, the supernatant was removed after centrifugation of the samples in the wells, and 150 μL of DMSO was added to dissolve the MTT formazan crystals. Finally, the absorbance was measured at 490 nm using a Bio-Rad microplate reader after shaking for 10 min and IC50 values were obtained from the graph of the DOX concentration versus the surviving cell viability. The cell viability was normalized to that of cells cultured with PBS treatment, which was served as the indicator of 100%.



Scheme 1. Schematic illustration of tumor suppression by synergistic treatment of CSCs with shSox2 and DOX.

2.4. qRT-PCR analysis of cancer cell-associated genes in CSCs

qRT-PCR analysis was further conducted to evaluate the expression of two cancer cell-associated genes (MDR1 and Sox2) between MCF-7 adherent cells and MCF-7 mammosphere cells. The propagated mammosphere cells were collected and the total mRNA was extracted from the cells with Trizol reagent according to the manufacturer's protocol. The concentration of total RNAs was measured using Nanodrop Plates (Tecan M200), and then the total RNAs were reverse transcribed into cDNA by using M-MLV manual (Promega). Finally, the qRT-PCR (Mx3005P, Strategene, USA) was performed using SYBR Green Mix Kit according to the manufacturer's protocol. The qRT-PCR amplification was carried out as follows: initial heating at 95 °C for 10 min, followed by 40 cycles at 95 °C for 30 s, 56 °C for 60 s, 72 °C for 60 s. The sequence of MDR1 primer forward: 5′-ATATCAGCAGCCCACATCAT-3′, reverse: 5′-GAAGCACTGGGATGTCCGGT-3'; The sequence of SOX2 primer forward: 5′-TCATTTGCCATCTGGATTTT-3′, reverse: 5′-CTCCTACCG-TACCACTAGAACTT-3'; The sequence of GAPDH primer forward: 5′-GTTCCAGTATGACTCTACCC-3′, reverse: 5′-AGTCTTCTGAGGCAGT-GATG-3'.

2.5. Gene transfection of CSCs

To evaluate the transfection efficiency of MCF-7 mammosphere cells, two commercial transfection reagents (PEI-25k and GoldenTransfer) were utilized to complex with the reporter gene *p*EGFP-N1 (Promega, Wisconsin, USA) and the GFP fluorescence was detected. Briefly, MCF-7 mammosphere cells were seeded in ultra-low attachment 96-well plates at 1.0×10^4 cells per well in 100 μL of serum-free DMEM-F12 at 37 °C in 5% CO_2 for 24 h. MCF-7 adherent cells were carried out as the control. PEI-25k/*p*EGFP-N1 or GoldenTransfer/*p*EGFP-N1 complex was added according to the manufacturer's protocol. The final concentration of *p*EGFP-N1 in the culture medium was 1 μg/mL. After incubation for another 48 h, the relative levels of EGFP expression were characterized by using fluorescence microscopy (Ti—S, Nikon, Japan).

2.6. Gene silence of CSCs

MCF-7 mammosphere cells were collected and seeded in ultra-low attachment 6-well plates at 2.0×10^5 cells per well in 2 mL of serum-free DMEM-F12 at 37 °C in 5% CO_2 for 24 h. MCF-7 adherent cells were used as the control. Small interfering RNA oligonucleotides (siMDR1) or the plasmid (shSox2) that can express small interfering RNA oligonucleotides were transfected using GoldenTransfer according to the manufacturer's protocols. The final concentration of gene in the culture medium was 1 μg/mL. After incubation for another 24 h, the propagated mammosphere cells were collected and the total mRNA was extracted from the cells with Trizol reagent according to the manufacturer's protocol. The concentration of total RNAs was measured using Nanodrop Plates, and then the total RNAs were reverse transcribed into cDNA by using M-MLV manual. Finally, the qRT-PCR was performed using SYBR Green Mix Kit according to the manufacturer's protocol.

2.7. Reversal of drug resistance of CSCs

To enhance the chemosensitivity of the conventional drugs for killing CSCs, RNAi gene silencing technology was introduced to knock down the expression of specific genes in CSCs before chemotherapy. Briefly, MCF-7 mammosphere cells and MCF-7 adherent cells were seeded in 6-well plates at 5.0×10^4 cells per well before 24 h, and then transfected using GoldenTransfer by silencing the cancer cell-associated genes (MDR1 or Sox2) according to the manufacturer's protocols. After incubation for 48 h, the cells were treated with DOX for another 4 h at a final concentration of 0.8 μg/mL. The cells were collected and washed twice with cold phosphate buffered saline (PBS, 0.01 M, pH 7.4). The cells were detached with 0.05% trypsin and resuspended in 300 μL cold PBS. Finally, the cellular uptake efficiency of DOX in the cancer cell-associated genes down-regulated cells was evaluated using a BD FACS Calibur flow cytometer (BD Bioscience, USA), MCF-7 adherent cells and MCF-7 mammosphere cells only treated with DOX were utilized as the controls.

2.8. Combination therapy of CSCs with shSox2 and DOX

The combination treatment of CSCs was further performed by silencing the over-expressed Sox2 gene by GoldenTransfer/shSox2 complex and inducing the cell apoptosis by DOX. Briefly, MCF-7 mammosphere cells were seeded in ultra-low attachment 6-well plates at 1.0×10^5 cells per well in 2 mL of serum-free DMEM-F12 at 37 °C in 5% CO_2 for 24 h. GoldenTransfer/shSox2 complex was added according to the manufacturer's protocol. The final concentration of shSox2 in the culture medium was 1 μg/mL. After incubation for 48 h, the transfected cells were collected and seeded in ultra-low attachment 96-well plates at 1.0×10^4 cells per well prior to the treatment of DOX at a concentration of 0.8 μg/mL. After another 48 h post-treatment, 20 μL of MTT stock solution was added to each well for 4 h, and then the supernatant was removed after centrifugation of the samples in the wells. 150 μL of DMSO was added to dissolve the MTT formazan crystals and the absorbance was measured at 490 nm using a Bio-Rad microplate reader after shaking for 10 min. The cell viability was normalized to that of cells cultured with PBS treatment, which was served as the indicator of 100%.

2.9. Statistical analysis

Statistical analysis was performed using the Student's t-test. All the data were given by a mean value together with its standard deviation (mean ± SD). If $P < 0.05$, the differences were regarded to be statistically significant.

3. Results and discussion

3.1. Formation of CSCs

CSCs are rare subpopulations of cancer cells, which cause cancer resistance and metastasis when treated with conventional chemotherapies [28]. Generally, CSCs can be obtained by fluorescence-activated cell sorting [29], rotary cell culture system [30], and cultivation in ultralow attachment plate [31]. For this study, the enriched CSCs were obtained by keeping MCF-7 cancer cells in suspension in the CSC culture medium. For this method, most non-CSCs become dead due to anoikis induced by the deprivation of attachment to substrate, while CSCs survive and proliferate to form CSC-enriched mammosphere cells in CSC medium [13]. As shown in Fig. 1, MCF-7 mammosphere cells were formed with the diameter of about 100 μm after 14 days of incubation, and was more luminous as compared with MCF-7 adherent cells. The subsequent generations of mammosphere cells were propagated from the single cells digested by the former mammosphere cells.

3.2. Drug resistant ability of CSCs

Currently, chemotherapy is still the most commonly used and effective method in clinical cancer therapy. However, chemotherapy usually leads to the drug resistance and recurrences with

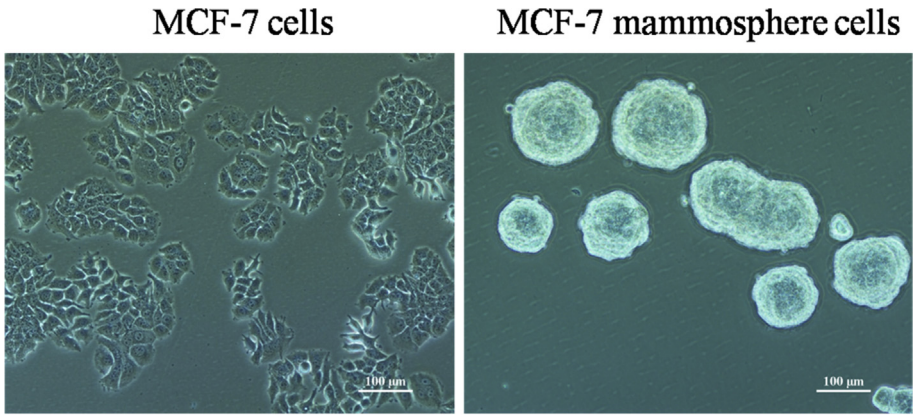


Fig. 1. Microscopic images of MCF-7 adherent cells and MCF-7 mammosphere cells (scale bar = 100 μm).

more distant metastases, even though it seems effective in the early-stage of treatment. CSCs are recognized to be responsible in the failure of chemotherapy due to their highly drug resistant and re-initiate the growth of tumors. To determine the drug resistant property of CSCs, MCF-7 adherent cells and freshly isolated MCF-7 mammosphere cells were subjected to the MTT assay, and DOX was performed as the conventional chemotherapy drug at various concentrations. The cytotoxicity was evaluated after 48 h incubation of DOX in two types of cells. As shown in Fig. 2, MCF-7 mammosphere cells were more resistant to DOX than MCF-7 adherent cancer cells, and the half-maximal inhibitory concentration (IC50) of the former (3.82 μg/mL) is 5.6 times than that of the latter (0.68 μg/mL). The results further validate the highly resistant ability of CSCs, and reversal of the drug resistance of CSCs should be an effective treatment strategy to enhance chemotherapy of cancer cells.

3.3. The expression of cancer cell-associated genes in CSCs

The role of tumor suppressor genes or stemness-associated genes would be crucial in CSCs genesis and metastasis, and these genes have been shown to be the fundamental factors in the acquisition of CSCs properties [20]. To evaluate the biological significance of the expression of cancer cell-associated genes (MDR1 and Sox2) between MCF-7 adherent cells and MCF-7 mammosphere cells, qRT-PCR analysis was carried out to evaluate the mRNA levels of the three former genes. As shown in Fig. 3, the cancer cell-associated genes MDR1 and Sox2 were up-regulated in MCF-7 mammosphere cells as compared with the control MCF-7 adherent cancer cells. These results further verify that the drug resistance and high metastatic ability of CSCs were mainly due to the up-regulation of some cancer cell-associated genes. Thus, down-regulation of some cancer cell-associated genes of CSCs is expected to contribute to cancer treatment.

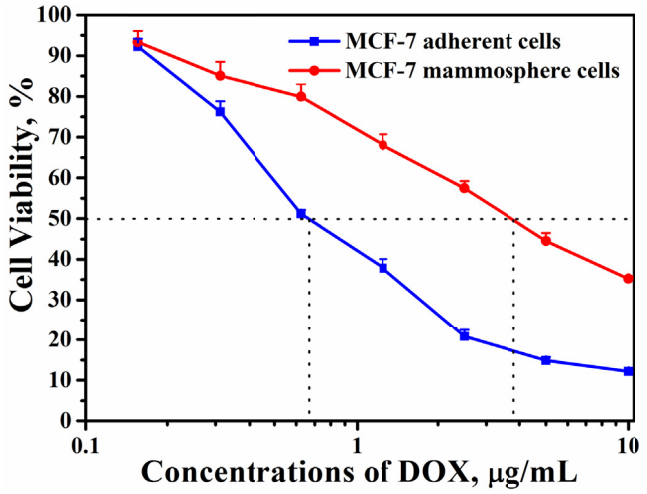


Fig. 2. The inhibition of cell proliferation results of MCF-7 adherent cells and MCF-7 mammosphere cells by DOX after 48 h (n = 4).

3.4. Gene transfection of CSCs

Transfection activities of MCF-7 adherent cells and MCF-7 mammosphere cells were examined by comparing the fluorescence intensities in fluorescence images by PEI-25k/*p*EGFP-N1 or GoldenTransfer/*p*EGFP-N1 complex. As shown in Fig. 4, as compared with PEI-25k, GoldenTransfer showed a significantly enhanced gene transfection efficiency in both MCF-7 adherent cells and MCF-7 mammosphere cells. For this reason, GoldenTransfer was chosen in subsequent studies. Although MCF-7 mammosphere cells did not show as much fluorescence intensities as that of MCF-7 adherent cells, it also showed impressive gene transfection efficiency by transfect reagents.

3.5. qRT-PCR analysis of gene silencing in CSCs

To evaluate the gene silencing activities of the proposed gene delivery system, the mRNA levels of MDR1 and Sox2 were measured in MCF-7 mammosphere cells. The final concentration of siRNA (or shRNA) in the culture medium was 1 μg/mL. The gene silencing efficiency was evaluated by quantitative analysis of the relative mRNA levels using qRT-PCR after 24 h treatment with GoldenTransfer/siMDR1 and GoldenTransfer/shSox2 complexes. MCF-7 mammosphere cells without any treatment were used as the control. As shown in Fig. 5, all the genes were significantly down-regulated, especially for the Sox2 gene (≈67%). It is anticipated that the effects of chemotherapeutic agents will be improved in CSCs after the cancer cell-associated genes were down-regulated.

3.6. Endocytosis of DOX to CSCs after gene silencing of cancer cell-associated genes

A combination therapeutic approach was carried out to CSCs to enhance the effect of chemotherapy. The cancer cell-associated

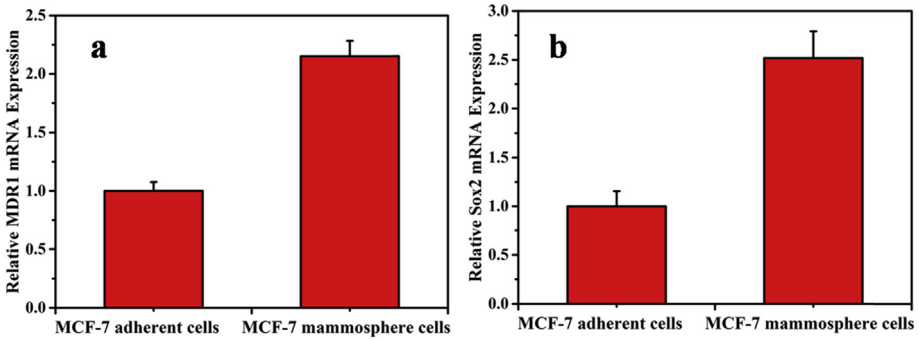


Fig. 3. Expression of cancer cell-associated genes in MCF-7 adherent cells and MCF-7 mammosphere cells. MDR1 (a) and Sox2 (b). Data are shown as means ± SD (n = 3).

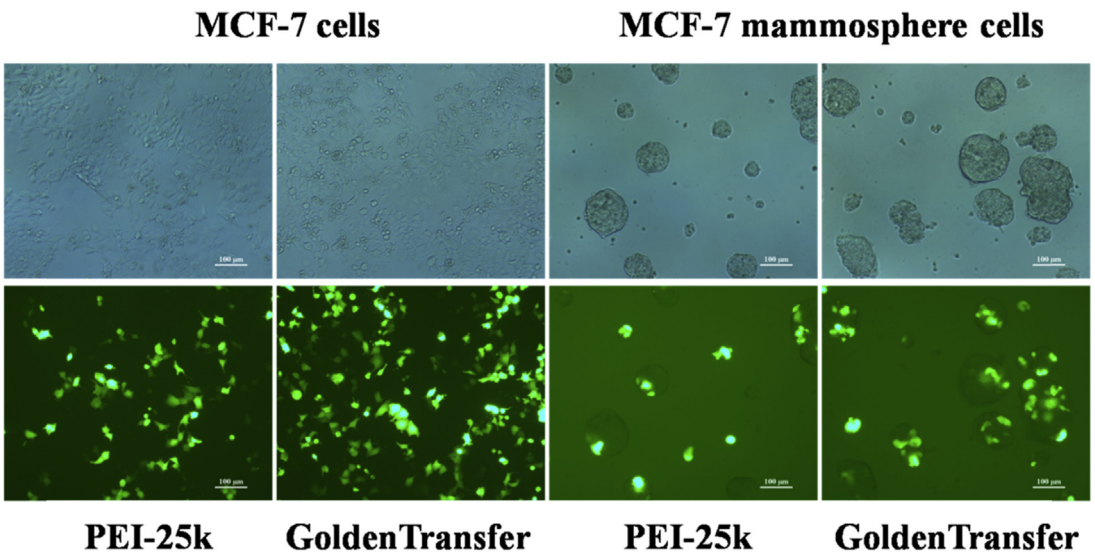


Fig. 4. The transfection efficiency shown by green fluorescent protein of MCF-7 adherent cells and MCF-7 mammosphere cells expressed by PEI-25k/*p*EGFP-N1 or GoldenTransfer/*p*EGFP-N1 complex (scale bar = 100 μm)(For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.).

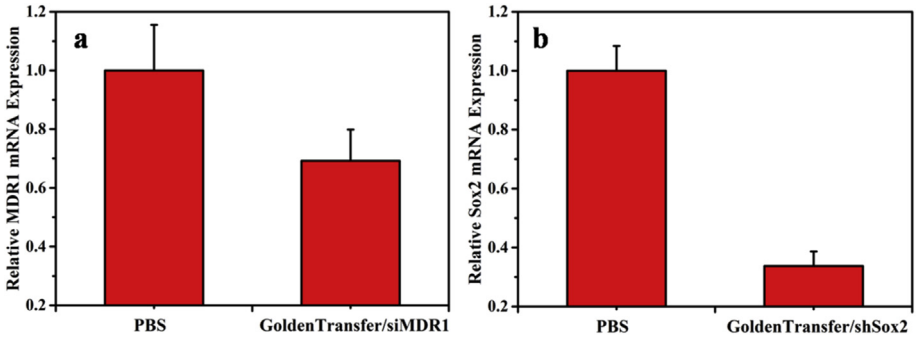


Fig. 5. qRT-PCR analysis for the mRNA of MDR1 (a) and Sox2 (b). MCF-7 mammosphere cells without any treatment were used as the controls, and the relative quantification was performed using GAPDH housekeeping gene as the internal control (n = 3).

genes (MDR1 and Sox2) were down-regulated for 48 h to reverse the drug resistance before delivering DOX for 4 h. As shown in Fig. 6, the endocytosis of DOX was improved after down-regulating the expression of MDR1 and Sox2 genes. Especially, the shSox2-treated group showed a more significant DOX endocytosis efficiency in MCF-7 mammosphere cells, which was close to the DOX endocytosis of MCF-7 adherent cancer cells (non-drug resistant cells). As compared with MCF-7 adherence cells, a much lower DOX endocytosis efficiency of MCF-7 mammosphere cells was achieved due to their drug resistance trait. These results further illustrate that the chemotherapy of CSCs can be significantly improved by introduction of the RNAi approach.

3.7. *The synergistic treatment of therapeutic shSox2 and DOX*

Chemotherapy is highly effective during the early-stage treatment of cancers. However, drug resistance and recurrences with more distant metastases will occur after repeating adminstration of

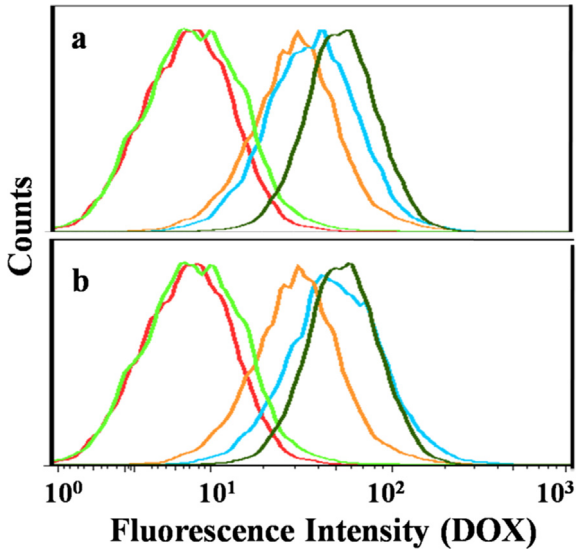


Fig. 6. Cellular uptake of DOX by MCF-7 adherent cells and MCF-7 mammosphere cells measured by flow cytometric analyses of DOX fluorescence after a 4 h endocytosis. MCF-7 mammosphere cells were transfected with GoldenTransfer/siMDR1 (a) and GoldenTransfer/shSox2 (b) for 48 h before adding DOX. Cells tested are MCF-7 adherent cells (red), MCF-7 mammosphere cells (light green), MCF-7 adherent cells with DOX (deep green), MCF-7 mammosphere cells with DOX (orange), and cancer cell-associated gene transfected MCF-7 mammosphere cells with DOX (blue)(For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.).

drugs [10]. The failure of chemotherapy is mainly attributed to the existence of CSCs [32]. A combined therapeutic strategy to tumors will be necessary due to their drug resistance trait and pluripotent properties [20,26]. Thus, targeting both CSCs and cancer cells with two kinds of drugs is one of the most effectively strategy in tumor treatment and prevention of relapse [11,32,33]. Reverse the cancer stem cell-associate genes is another potential strategy to enhance the efficacy of chemotherapy [34]. From this perspective, RNAi approach was carried out to improve the effect of DOX in this study. To verify whether the combined therapeutic strategy of Sox2 gene silencing and DOX could effectively inhibit the tumor growth of CSCs, the stem cell-associated gene Sox2 was down-regulated for 48 h to reverse the drug resistance, and then DOX was added to the cells for another 48 h. As shown in Fig. 7, the cell viability of GoldenTransfer/shSox2 and GoldenTransfer/shNC groups didn't show significantly differences compared with PBS control group. The cell survival rate of the free DOX treated group was about 75.2%, which achieved a comparative anti-proliferation results as compare with the combinational therapeutic of GoldenTransfer/shNC and DOX group (73.8%). For the combined therapeutic of GoldenTransfer/shSox2 and DOX group, a synergistic therapeutic effect was obtained at the final cell survival rate of 46.8% in MCF-7 mammosphere cells. To our great surprise, the cell survival rate could only achieve about 45% in MCF-7 adherent cells by only DOX treated group at the same DOX concentration (data were not shown). The results further demonstrated that the drug resistance of CSCs was almost reversed through down-regulation of Sox2 gene.

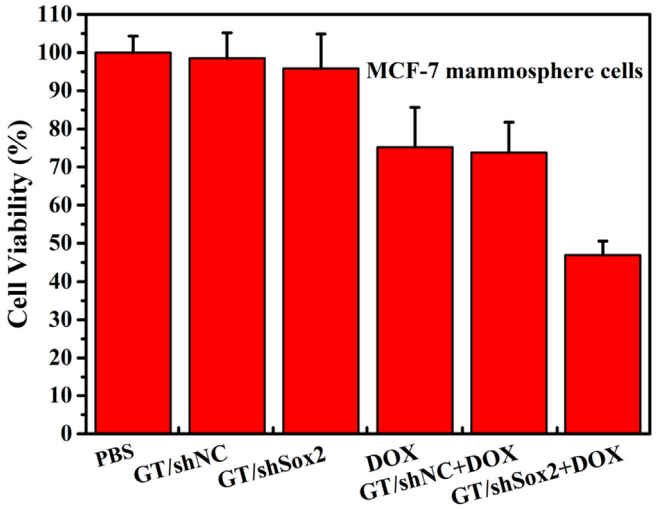


Fig. 7. The combined treatment of therapeutic shSox2 and DOX in MCF-7 mammosphere cells. GT is short for GoldenTransfer reagent. Data are shown as mean ± SD (n = 4).

4. Conclusions

In this study, a new strategy for the treatment of CSCs was developed by combining antioncogenes and DOX. Two cancer cell-associated genes (MDR1 and Sox2) were shown to be up-regulated in CSCs, which exhibited more drug resistance as compared with normal adherent cancer cells. RNAi technology was successfully used to evaluate the gene silencing effect of CSCs by PEI-25k and GoldenTransfer reagents. The multidrug resistance of CSCs could be reversed by down regulation of the over-expressed cancer cell-associated MDR1 and Sox2 genes, thus the enhanced DOX endocytosis was achieved after down-regulation of the genes. In addition, a combined treatment of shSox2 and DOX was carried out to overcome the obstacles of the drug resistance of CSCs, and an enhanced antitumor therapy was achieved. The combination therapy of down-regulating CSCs' multidrug resistance and a therapeutic antitumor drug can be a potential strategy in cancer treatment.

Conflict of interest

The authors report no declarations of interest.

Acknowledgements

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References

[1] D. Peer, J.M. Karp, S. Hong, O.C. FaroKHzad, R. Margalit, R. Langer, Nanocarriers as an emerging platform for cancer therapy, Nat. Nanotechnol. 2 (12) (2007) 751–760.
[2] S.K. Das, M.E. Menezes, S. Bhatia, X.Y. Wang, L. Emdad, D. Sarkar, P.B. Fisher, Gene therapies for cancer: strategies, challenges and successes, J. Cell. Physiol. 230 (2) (2015) 259–271.
[3] N.S. Gandhi, R.K. Tekade, M.B. Chougule, Nanocarrier mediated delivery of siRNA/miRNA in combination with chemotherapeutic agents for cancer therapy: current progress and advances, J. Control. Release 194 (2014) 238–256.
[4] U.H. Jeong, V.K. Garripelli, S. Jo, C.S. Myung, S.J. Hwang, J.K. Kim, J.S. Park, Potential of pH-sensitive polymer-anchored cationic liposomes for combinatorial anticancer therapy with doxorubicin and siRNA, J. Drug Deliv. Sci. Technol. 24 (1) (2014) 27–32.
[5] J. Chen, Z.X. Jiao, L. Lin, Z.P. Guo, C.N. Xu, Y.H. Li, H.Y. Tian, X.S. Chen,

Polylysine-modified polyethylenimines as siRNA carriers for effective tumor treatment, Chin. J. Polym. Sci. 33 (6) (2015) 830–837.
[6] H.Y. Tian, Z.P. Guo, L. Lin, Z.X. Jiao, J. Chen, S.Q. Gao, X.J. Zhu, X.S. Chen, pH-responsive zwitterionic copolypeptides as charge conversional shielding system for gene carriers, J. Control. Release 174 (2014) 117–125.
[7] P. Bhardwaj, R. Singh, A. Swarup, Development and characterization of newer floating film bearing 5-fluorouracil as a model drug, J. Drug Deliv. Sci. Technol. 24 (5) (2014) 486–490.
[8] Z.X. Jiao, J. Chen, H.Y. Tian, X.S. Chen, Synergistic antitumor effect of cisplatin and siRNA mediated by polyethylenimine, Acta Polym. Sin. 1 (2015) 127–132.
[9] X.W. Guan, Y.H. Li, Z.X. Jiao, L.L. Lin, J. Chen, Z.P. Guo, H.Y. Tian, X.S. Chen, Codelivery of antitumor drug and gene by a pH-sensitive charge-conversion system, ACS Appl. Mater. Interfaces 7 (5) (2015) 3207–3215.
[10] M.K. Asiedu, F.D. Beauchamp-Perez, J.N. Ingle, M.D. Behrens, D.C. Radisky, K.L. Knutson, AXL induces epithelial-to-mesenchymal transition and regulates the function of breast cancer stem cells, Oncogene 33 (10) (2014) 1316–1324.
[11] X.Y. Ke, V.W.L. Ng, S.J. Gao, Y.W. Tong, J.L. Hedrick, Y.Y. Yang, Co-delivery of thioridazine and doxorubicin using polymeric micelles for targeting both cancer cells and cancer stem cells, Biomaterials 35 (3) (2014) 1096–1108.
[12] S.S. Kim, A. Rait, F. Rubab, A.K. Rao, M.C. Kiritsy, K.F. Pirollo, S. Wang, L.M. Weiner, E.H. Chang, The clinical potential of targeted nanomedicine: delivering to cancer stem-like cells, Mol. Ther. 22 (2) (2014) 278–291.
[13] W. Rao, S.T. Zhao, J.H. Yu, X.B. Lu, D.L. Zynger, X.M. He, Enhanced enrichment of prostate cancer stem-like cells with miniaturized 3D culture in liquid core-hydrogel shell microcapsules, Biomaterials 35 (27) (2014) 7762–7773.
[14] D. Bonnet, J.E. Dick, Human acute myeloid leukemia is organized as a hierarchy that originates from a primitive hematopoietic cell, Nat. Med. 3 (7) (1997) 730–737.
[15] S.E. Mathis, A. Alberico, R. Nande, W. Neto, L. Lawrence, D.R. McCallister, J. Denvir, G.A. Kimmey, M. Mogul, G. Oakley, K.L. Denning, T. Dougherty, J.V. Valluri, P.P. Claudio, Chemo-predictive assay for targeting cancer stem-like cells in patients affected by brain tumors, Plos One 9 (8) (2014).
[16] T.M. Sun, Y.C. Wang, F. Wang, J.Z. Du, C.Q. Mao, C.Y. Sun, R.Z. Tang, Y. Liu, J. Zhu, Y.H. Zhu, X.Z. Yang, J. Wang, Cancer stem cell therapy using doxorubicin conjugated to gold nanoparticles via hydrazone bonds, Biomaterials 35 (2) (2014) 836–845.
[17] E.N. Garza-Trevino, S.L. Said-Fernandez, H.G. Martinez-Rodriguez, Understanding the colon cancer stem cells and perspectives on treatment, Cancer Cell. Int. 15 (1) (2015) 2–9.
[18] Y. Yang, J.J. Hou, Z. Lin, H. Zhuo, D.Y. Chen, X.D. Zhang, Y. Chen, B.C. Sun, Attenuated listeria monocytogenes as a cancer vaccine vector for the delivery of CD24, a biomarker for hepatic cancer stem cells, Cell. Mol. Immunol. 11 (2) (2014) 184–196.
[19] H.X. Zhan, J.W. Xu, D. Wu, T.P. Zhang, S.Y. Hu, Pancreatic cancer stem cells: new insight into a stubborn disease, Cancer Lett. 357 (2) (2015) 429–437.
[20] J.A. Leal, M.E. Lleonart, MicroRNAs and cancer stem cells: therapeutic approaches and future perspectives, Cancer Lett. 338 (1) (2013) 174–183.
[21] J. Miki, B. Furusato, H.Z. Li, Y.P. Gu, H. Takahashi, S. Egawa, I.A. Sesterhenn, D.G. McLeod, S. Srivastava, J.S. Rhim, Identification of putative stem cell markers, CD133 and CXCR4, in hTERT-immortalized primary nonmalignant and malignant tumor-derived human prostate epithelial cell lines and in prostate cancer specimens, Cancer Res. 67 (7) (2007) 3153–3161.
[22] B. Sharpe, M. Beresford, R. Bowen, J. Mitchard, A.D. Chalmers, Searching for prostate Cancer stem cells: markers and methods, Stem Cell Rev. Reports 9 (5) (2013) 721–730.
[23] P.B. Gupta, T.T. Onder, G.Z. Jiang, K. Tao, C. Kuperwasser, R.A. Weinberg, E.S. Lander, Identification of selective inhibitors of cancer stem cells by high-throughput screening, Cell 138 (4) (2009) 645–659.
[24] L.V. Nguyen, R. Vanner, P. Dirks, C.J. Eaves, Cancer stem cells: an evolving concept, Nat. Rev. Cancer 12 (2) (2012) 133–143.
[25] E. Sachlos, R.M. Risueno, S. Laronde, Z. Shapovalova, J.H. Lee, J. Russell, M. Malig, J.D. McNicol, A. Fiebig-Comyn, M. Graham, M. Levadoux-Martin, J.B. Lee, A.O. Giacomelli, J.A. Hassell, D. Fischer-Russell, M.R. Trus, R. Foley, B. Leber, A. Xenocostas, E.D. Brown, T.J. Collins, M. Bhatia, Identification of drugs including a dopamine receptor antagonist that selectively target cancer stem cells, Cell 149 (6) (2012) 1284–1297.
[26] M.T. Mueller, P.C. Hermann, J. Witthauer, B. Rubio-Viqueira, S.F. Leicht, S. Huber, J.W. Ellwart, M. Mustafa, P. Bartenstein, J.G. D'Haese, M.H. Schoenberg, F. Berger, K.W. Jauch, M. Hidalgo, C. Heeschen, Combined targeted treatment to eliminate tumorigenic cancer stem cells in human pancreatic cancer, Gastroenterology 137 (3) (2009) 1102–1113.
[27] C.X. Liu, G. Zhao, J. Liu, N.C. Ma, P. Chivukula, L. Perelman, K. Okada, Z.Y. Chen, D. Gough, L. Yu, Novel biodegradable lipid nano complex for siRNA delivery significantly improving the chemosensitivity of human colon cancer stem cells to paclitaxel, J. Control. Release 140 (3) (2009) 277–283.
[28] J.E. Dick, Looking ahead in cancer stem cell research, Nat. Biotechnol. 27 (1) (2009) 44–46.
[29] Z.L. Yang, Q. Zheng, J. Yan, Y. Pan, Z.G. Wang, Upregulated CD133 expression in tumorigenesis of colon cancer cells, World J. Gastroenterol. 17 (7) (2011) 932–937.
[30] S.E. Kelly, A. Di Benedetto, A. Greco, C.M. Howard, V.E. Sollars, D.A. Primerano, J.V. Valluri, P.P. Claudio, Rapid selection and proliferation of CD133(+) cells from cancer cell lines: chemotherapeutic implications, Plos One 5 (4) (2010).
[31] R. Sun, Y. Liu, S.Y. Li, S. Shen, X.J. Du, C.F. Xu, Z.T. Cao, Y. Bao, Y.H. Zhu, Y.P. Li, X.Z. Yang, J. Wang, Co-delivery of all-trans-retinoic acid and doxorubicin for cancer therapy with synergistic inhibition of cancer stem cells, Biomaterials 37 (2015) 405–414.
[32] Y. Zhou, J.Y. Yang, J.S. Rhim, J. Kopecek, HPMA copolymer-based combination therapy toxic to both prostate cancer stem/progenitor cells and differentiated cells induces durable anti-tumor effects, J. Control. Release 172 (3) (2013) 946–953.
[33] A. Dubrovska, J. Elliott, R.J. Salamone, S. Kim, L.J. Aimone, J.R. Walker, J. Watson, M. Sauveur-Michel, C. Garcia-Echeverria, C.Y. Cho, V.A. Reddy, P.G. Schultz, Combination therapy targeting both tumor-initiating and differentiated cell populations in prostate carcinoma, Clin. Cancer Res. 16 (23) (2010) 5692–5702.
[34] M. Piva, G. Domenici, O. Iriondo, M. Rabano, B.M. Simoes, V. Comaills, I. Barredo, J.A. Lopez-Ruiz, I. Zabalza, R. Kypta, M.D.M. Vivanco, Sox2 promotes tamoxifen resistance in breast cancer cells, Embo Mol. Med. 6 (1) (2014) 66–79.